bjh short report

High-density mapping and follow-up studies on chromosomal regions 1, 3, 6, 12, 13 and 17 in 28 families with chronic lymphocytic leukaemia

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Summary

A subset of chronic lymphocytic leukaemia (CLL) shows familial aggregation. Studies show an increased risk for CLL and other lymphoproliferative disease among first-degree relatives of affected individuals. A genome-wide scan of 18 CLL families in 2003 detected LOD or non-parametric linkage scores ≥1·0 on chromosomes 1, 3, 6, 12, 13 and 17. Follow-up study with 28 families showed no evidence of linkage at 1p22.1–p21.2, 3q22.1, 3q26.2, 6q22.31–q23.2, 12q24.23, 14q32.13, 17p13.3. Chromosome 13q21.33 remains a region of interest with a *P*-value of 0·013 (marker D13S1291) and warrants additional molecular investigation as a susceptibility region for CLL.

Keywords: chronic lymphocytic leukaemia, family studies, linkage analysis.

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There is evidence that a subset of chronic lymphocytic leukaemia (CLL) is due to genetic susceptibility. Over 80 families have been reported to show familial aggregation of CLL (Marti *et al*, 2003). Population and family studies showed a sevenfold increased risk of CLL and twofold increased risk for Hodgkin lymphoma among first-degree relatives of patients with CLL (Goldin *et al*, 2004). A causative factor has not been identified for familial CLL. Since 1974, the Genetic Epidemiology Branch (Division of Cancer Epidemiology and Genetics, National Institutes of Health) has enrolled families with two or more living cases of CLL. To identify genetic susceptibility loci for familial CLL, we performed a detailed mapping study of the chromosomal regions identified by a whole genome scan (Goldin *et al*, 2003) with 28 families.

Patients and methods

Ascertainment of CLL pedigrees

This study was approved by an institutional review board and informed consent was obtained from all subjects included in this report. CLL diagnoses were documented by history, review of medical records, physical examination and pathological review of slides. Twenty-eight families were judged to be informative for linkage studies based on the availability of DNA samples from affected and unaffected individuals (63 affected, total 155 individuals). Sixteen families were previously analysed with a whole genome scan (Goldin *et al*, 2003).

Genotyping

DNA was extracted from peripheral blood or cryopreserved lymphocytes. Genotyping was performed by deCODE Genetics. Microsatellite markers ($n = 102$, average heterozygosity 74%) spaced *c.*1 Mb apart were selected from the deCODE panel to genotype patients up to a distance of 10 Mb flanking the centromeric and telomeric regions around markers (D1S2868, D1S206, D3S1292, D3S1614, D6S287, D6S262, D12S86, D13S156 and D17S849) identified as regions of interest from a whole genome scan performed on 18 CLL families (Goldin *et al*, 2003). Two markers were selected to flank *TCL1* at 14q32.13 to determine if any of the families

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contained affected individuals who co-segregated with this candidate gene. *TCL1* is a proto-oncogene that produces B-cell lymphomas in mice when overexpressed (Bichi *et al*, 2002). Marker positions were based on the deCODE genetic map (Kong *et al*, 2002). Allele frequencies for each marker were calculated from the cohort genotype data using all individuals.

Statistical analysis

Genotyping data were checked for errors with PEDCHECK (O'Connell & Weeks, 1998), MERLIN (Abecasis *et al*, 2002) and SimWalk2 (Sobel *et al*, 2002). In total, <1% of genotypes were eliminated because of Mendelian inconsistencies or high mistyping probability. Parametric and non-parametric (model-free) multipoint linkage analyses were conducted using GENEHUNTER, version 2.1 r5 beta (Kruglyak *et al*, 1996). Dominant and recessive models of inheritance were used in combination with a 'narrow' (CLL only as affected) or 'broad' [CLL and individuals with monoclonal B-cell lymphocytosis (MBL) as affected] definition of disease for parametric analyses. CLL allele frequencies, penetrance and liability classes were identical to values used in the whole genome scan (Goldin *et al*, 2003). Penetrance for the normal genotypes was set at 0·001 to allow for phenocopies. A total of four unaffected individuals were dropped from the GENEHUNTER analysis because of computational constraints. As a check on the possible loss of information content secondary to dropped individuals from the GENEHUNTER analyses, non-parametric linkage scores were also calculated using the SimWalk2 program v. 2·89 (Sobel & Lange, 1996).

Table I. SimWalk2 NPL scores chromosome 13.

Marker	Location (cM)	−Log10(p)	*P*-value
D13S1308	65·12	1·65	0·022
D13S1291	66·98	1·90	0·013
D13S152	69·02	1·80	0·016
D13S156	71·45	1·86	0·014
D13S792	71·83	1·82	0·015
D13S1306	75·05	1·54	0·029

Results

For chromosome 13q21.33–q22.3 markers, GENEHUNTER non-parametric linkage scores were positive, but not significant. However, statistics derived from SimWalk2 had *P*-values <0·05 for six adjacent markers (Table I). GENEHUNTER computes exact multipoint linkage statistics, but has limits on the size of the pedigrees. The SimWalk2 program can accommodate large pedigrees and simulates an approximate multipoint linkage statistic. Therefore, linkage scores generated from each program are often similar, but not identical. GENEHUNTER parametric and non-parametric linkage scores derived from dense microsatellite genotype data did not support evidence of linkage to chromosome regions 1p, 3q, 6q, 12q and 17p (Table II) under dominant or recessive inheritance and narrow (CLL only) affection status model. Parametric and non-parametric statistics generated for the broad (CLL and MBL) definition of affection status was negative (data not shown). The current data set did not identify regions of linkage based on heterogeneity as evidenced by non-significant heterogeneity LOD alpha scores (data not shown). There was no evidence to support linkage to *TCL1* at 14q32.13 (data not shown).

Discussion

Non-parametric linkage scores remained nominally significant ($P \leq 0{\cdot}029$) across six adjacent markers (Table I) spanning a distance of 6 Mb in region 13q21.33–q22.3. The peak signal was at marker D13S1291 ($P = 0{\cdot}013$). However, these linkage scores were not statistically significant in the context of a whole genome scan. Region 13q21.33 is situated 20 Mb telomeric to 13q14 the most frequent deletion found in CLL tumour cells.

Table II. Comparison of LOD or NPL score from the whole genome scan of 18 families and follow-up detailed mapping study with 28 families under the 'narrow' affection status model.

Marker(s)	Chromosome	Model	LOD or NPL WGS 18 families	*P*-value of WGS 18 families	Lod or NPL 28 families	*P*-value of 28 families
D1S2868-D1S206	1	Dom	1·32	0·006	−13·27	
D3S1292	3	Rec	1·51*	0·016	−15·56	
D3S1292	3	NPL	1·63	0·038	−0·02	0·5
D3S1614	3	NPL	1·68	0·034	1·21	0·1
D6S287-D6S262	6	Dom	1·23	0·008	−11·31	
D12S86	12	Dom	1·27	0·007	−6·39	
D12S86	12	NPL	2·81	0·002	1·41	0·07
D13S156	13	NPL	1·78	0·027	0·99	0·15 (0·014)
D17S849	17	NPL	2·78	0·003	1·024	0·14

Whole genome scan (WGS) parametric and non-parametric linkage (NPL) scores were calculated using GENEHUNTER PLUS. Detailed mapping studies with 28 families parametric and NPL scores were calculated with GENEHUNTER and Simwalk2.
*LOD score assuming heterogeneity.

Somatic deletion of 13q21–q22 has been observed in Scandinavian breast cancer families (Kainu *et al*, 2000), but to our knowledge this region not been previously studied in familial CLL. A detailed mapping study of 28 CLL families did not support linkage to chromosome regions 1p, 3q, 6q, 12q and 17p initially identified as positive on a whole genome scan. Genetic predisposition to familial CLL is most probably due to a heterogeneous collection of susceptibility loci (Sellick *et al*, 2005). Loss of regions 13q21–q22 have been observed in familial breast cancer and warrants additional studies to determine if a mutual tumour suppressor gene may predispose to familial CLL.

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